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Indian Standard

TOBACCO AND TOBACCO PRODUCTS — METHODS FOR DETERMINATION OF REDUCING SUGARS AND SUCROSE

(First Revision)

भारतीय मानक

तम्बाकू और तम्बाकू उत्पाद — अपचायक शक्कर और सुक्रोज ज्ञात करने की पद्धति

(पहला पुनरीक्षण)

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BUREAU OF INDIAN STANDARDS
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NEW DELHI 110002

September 1989 **Price Group 2**

Tobacco and Tobacco Products Sectional Committee, AFDC 13

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards on 10 January 1989, after the draft finalized by the Tobacco and Tobacco Products Sectional Committee had been approved by the Agricultural and Food Products Division Council.

The reducing sugars and sucrose content of tobacco is the indication of the quality of raw material. This standard will help in getting uniform and reproducible results while determining the reducing sugars and sucrose in tobacco and tobacco products.

This standard was originally issued in 1978 and at that time, considerable assistance was derived from an ISO proposal on the subject.

This revision has been taken up in order to update the standard in the light of current industrial and trade practices.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

AMENDMENT NO. 1 AUGUST 2005
TO

IS 8846 : 1989 TOBACCO AND TOBACCO PRODUCTS — METHODS FOR DETERMINATION OF REDUCING SUGAR AND SUCROSE

(First Revision)

(*Page* 1, *clause* **2**) — Substitute the following for the existing text:

'The following standard contains provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standard indicated below:

'IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)'

(*Page* 1, *clause* **4**, *line* 2) — Substitute 'IS 1070' *for* 'IS 1070 : 1977'.

(*Page* 1, *clause* **5.4**) — Substitute the following for the existing text:

'**5.4 Filter Paper**, medium porosity or Whatman No. 40 or its equivalent.'

(*Page* 2, *clause* **8.3.1**, *first para*) — Substitute the following for the existing text:

'Add exactly 100 ml distilled water to the Erlenmeyer flask (*see* clause **8.2**) and shake for one hour.'

(FAD 4)

Reprography Unit, BIS, New Delhi, India

Indian Standard

TOBACCO AND TOBACCO PRODUCTS — METHODS FOR DETERMINATION OF REDUCING SUGARS AND SUCROSE

(First Revision)

1 SCOPE

This standard lays down method for determination of reducing sugars and sucrose content in tobacco and tobacco products.

2 REFERENCE

IS 1070 : 1977 Specification for water for general laboratory use (*second revision*) is a necessary adjunct to this standard.

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Reducing Sugars

The amount of carbohydrate extracted from the sample and determined directly on the extract according to the procedure described in this standard.

3.2 Reducing Sugars and Sucrose

The amount of carbohydrate extracted from the sample and determined on the extract after inversion by acid hydrolysis according to the procedure described in this standard.

4 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070 : 1977) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

5 APPARATUS

5.1 Water Bath

5.2 Test Tubes, Light Walled, 200 mm length × 32 mm outer diameter.

5.3 Glass Bulbs, blown from heavy walled glass tubing and of such a size as to be supported on the rim of the test tube.

5.4 Filter Paper

5.5 Burettes

5.6 Bulb Pipettes, 5, 10, 20, 25, and 50-ml capacities.

5.7 Hot Plate

5.8 Water Bath, Fast Boiling, with test tube metal rack.

5.9 Laboratory Mill

6 REAGENTS

6.1 Lead Acetate Solution, saturated, neutral.

6.2 Copper — Iodometric Micro Reagent, containing:

Sodium carbonate, anhydrous	25 g
Potassium sodium tartarate (Rochelle salt)	25 g
Cupric sulphate solution, 10 percent	75 ml
Sodium hydrogen carbonate, anhydrous	20 g
Potassium iodide	5 g
Potassium iodate solution, 0·1 N	200 ml

6.2.1 *Preparation of Micro Reagent*

Dissolve sodium carbonate and Rochelle salt in 500 ml of distilled water (without heating). Add 75 ml of the cupric sulphate solution slowly by pipette well below the surface of the liquid and stir the solution. Add the sodium hydrogen carbonate and dissolve by stirring, and then add potassium iodide. Add 200 ml of the potassium iodate solution and dilute to one litre in a volumetric flask. Leave for 2 days. Filter the solution and keep in an amber-coloured reagent bottle. The reagent will be stable for one year.

6.3 Sodium Thiosulphate Solution, 0·1 N, prepared as follows.

6.3.1 *Preparation*

Dissolve 25 g of sodium thiosulphate in distilled water and dilute to one litre.

6.3.2 *Standardization*

Standardize the sodium thiosulphate solution against 0·1 N potassium iodate solution. For titration, take 25 ml potassium iodate, 3 g potassium iodide, 10 ml of 1 N sulphuric acid. Add 2 ml of 1 percent fresh starch solution as indicator (**6.4**) and dilute with 150 ml of water just before yellow coloured end point is reached.

> NOTE — 0·1 N sodium thiosulphate solution is quite stable but should be checked at least once in a month for its strength.

6.3.3 *Sodium Thiosulphate Solution*, 0·005 N.

Dilute the proper volume of 0·1 N sodium thiosulphate solution to 500 ml. This volume is calculated by dividing 2·5 by the normality of the standard thiosulphate solution. This volume can conveniently be measured by burette and need only be accurate to the nearest 0·05 ml. The 0·005 N sodium thiosulphate solution is unstable and should be discarded after 4 hours.

6.4 Starch Indicator Solution

Add 1 g soluble starch in 20 ml water and pour into about 80 ml boiling water. Boil for one minute and cool. A fresh starch solution is preferred for titration.

6.5 Phenolphthalein Indicator Solution, 0·5 percent.

Dissolve 5 g phenolphthalein in 500 ml ethanol and add 500 ml water with constant stirring. Filter if a precipitate forms.

6.6 Sodium Hydroxide Solution, 0·1 and 0·5 N.

6.7 Sulphuric Acid Solution, 0·1 and 1 N.

6.8 Hydrochloric Acid, 0·5 N.

6.9 Disodium Hydrogen Phosphate, analytical reagent grade.

6.10 Potassium Oxalate Monohydrate, analytical reagent grade.

7 PRINCIPLE

7.1 Reducing Sugars

Extract soluble carbohydrates from the ground sample with water for one hour. Remove the interfering materials with lead acetate. Determine the reducing sugars by the Shaffer-Somegyi-Hartmann copper-iodometric method.

7.2 Reducing Sugars and Sucrose

Extract soluble carbohydrates from the ground sample with water for one hour and inverse the sucrose by acid hydrolysis. Determine the sugars by the Shaffer-Somegyi-Hartmann copper-iodometric method.

8 PROCEDURE

8.1 Preparation of Sample

Air dry the whole leaf or cut leaf tobacco sample to a moisture content of between 8 and 12 percent and pass it through a suitable cutting mill fitted with a screen of nominal aperture size of one millimetre. Remove any filter or wrapping paper from tobacco products and proceed as above.

8.1.1 Mix thoroughly the ground sample preferably by mechanical means. If the sample is not to be examined at once, store in a wide-mouthed air-tight container of such a capacity that the sample may be adequately mixed by inverting the container before taking any sample for analysis.

8.2 Test Portion

Weigh accurately to the nearest 0·01 g in duplicate about 1 g of the prepared sample in a 6-ml glass weighing scoop and transfer into a 250-ml Erlenmeyer flask. Re-weigh the empty scoop.

8.3 Extraction

8.3.1 *Reducing Sugar and Sucrose*

Add exactly 100 ml of distilled water and shake for one hour.

8.4 Removal of Interfering Substances

8.4.1 *Precipitation with Lead Acetate*

To the solution from **8.3.1** add 3 ml of the lead acetate solution. Swirl to mix and allow the precipitate to settle.

8.4.2 *Deleading*

Filter the contents of the flask from **8.4.1** into a clean dry 50-ml conical flask containing 1 g potassium oxalate and 3 g disodium hydrogen phosphate and collect about 40 ml of filtrate. Swirl the contents of the flask and allow the precipitate to settle for one hour and filter into a clean and dry conical flask through a dry medium porosity filter paper.

8.5 Inversion of Sucrose

Take with a pipette, 10 ml of solution from **8.4.2** into a 100-ml volumetric flask, add 5 ml of 0·5 N hydrochloric acid and heat in a boiling water-bath for 30 minutes. Cool and add 5 ml of 0·5 N sodium hydroxide solution. Add 3 drops of phenolphthalein and neutralize, if necessary, with either 0·1 N sodium hydroxide or 0·1 N sulphuric acid solutions depending on the acidity or alkalinity of the solution and make up the volume.

8.6 Determination of Sugar Content

8.6.1 *Reducing Sugar*

Take with a pipette, 10 ml of solution from **8.4.2** into a 100-ml volumetric flask and add 3 drops of phenolphthalein. If necessary, add by a burette 0·1 N sodium hydroxide solution to the contents of the volumetric flask until slightly pink. Neutralize with 0·1 N sulphuric acid solution if it is too alkaline.

8.6.2 Pipette accurately 5 ml aliquot from **8.6.1** (for reducing sugars) and 5 ml aliquot from **8.5** for reducing sugar and sucrose, as the case may be, into a test tube and add accurately 5 ml of the copper-iodometric-micro-reagent using the micro-reagent to rinse the sugar solution from the walls of the test tube. Shake the tube gently to mix and stopper with a glass bulb to prevent atmospheric oxidation during heating and cooling. Place the tube for 20 minutes vertically in a vigorously boiling water bath equipped with metal rack. The rack should hold the tube snugly to avoid agitation during boiling. Remove the rack with tube and cool to 35°C in a constant temperature water bath, alternatively cool under cold tap water (30 to 35°C). Avoid agitation whilst the solution is still alkaline. Add 5 to 6 ml of 1 N sulphuric acid by pipette, rinsing the walls of the tube. Shake vigorously. Allow to stand for 10 to 15 minutes in the water-bath with occasional shaking (about 3 times during this period). Titrate with sodium thiosulphate solution, using 1 ml starch indicator solution towards the end (when the solution becomes light yellow). Prepare three blanks by using 5 ml distilled water instead of sugar solution. Heat, acidify and titrate against the sodium thiosulphate solution. It may not be necessary to do blanks every day since the micro-reagent is stable. A weekly check should suffice.

9 CALCULATION

9.1 The blank titration minus the sample titration gives the volume of 0·005 N sodium thiosulphate equivalent to the sugar in 5 ml sample.

9.2 Calculate the milligrams of glucose equivalent to the titration difference from Table 1 and calculate the percentage of sugars as follows:

$$\text{Sugars (as glucose), percent by dry mass} = \frac{G \times 2\,060}{M\,(100 - m)}$$

where

G = glucose in mg (*see* Table 1),

M = mass of the sample in g, and

m = percent moisture.

9.2.1 In the event, the titre value exceeds 17·9 ml, the following formula may be used for converting the titre value into mg glucose:

$$\text{Glucose, mg} = 0{\cdot}112 \text{ (ml of } 0{\cdot}005 \text{ N sodium thiosulphate)} + 0{\cdot}04$$

Table 1 Milligrams of Glucose Corresponding to the Difference Between the Titration Values for the Blank and the Unknown

(*Clause* 9.2)

0·005 N $Na_2S_2O_3$	Milligram of Glucose Corresponding to Titration Difference of									
	0·0	0·1	0·2	0·3	0·4	0·5	0·6	0·7	0·8	0·9
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
ml										
0			0·05	0·06	0·08	0·09	0·10	0·11	0·13	0·14
1	0·15	0·16	0·17	0·19	0·20	0·21	0·22	0·21	0·24	0·35
2	0·27	0·28	0·29	0·30	0·31	0·32	0·33	0·35	0·36	0·37
3	0·38	0·39	0·41	0·41	0·42	0·43	0·45	0·46	0·47	0·48
4	0·49	0·50	0·53	0·54	0·54	0·55	0·56	0·57	0·58	0·59
5	0·60	0·61	0·64	0·65	0·65	0·66	0·67	0·68	0·69	0·70
6	0·72	0·73	0·74	0·75	0·76	0·77	0·78	0·79	0·80	0·82
7	0·83	0·84	0·85	0·86	0·87	0·88	0·89	0·90	0·91	0·83
8	0·94	0·95	0·96	0·97	0·98	0·99	1·00	1·01	1·02	1·04
9	1·05	1·06	1·07	1·08	1·09	1·10	1·11	1·12	1·13	1·15
10	1·16	1·17	1·18	1·19	1·20	1·21	1·22	1·23	1·24	1·26
11	1·27	1.28	1·29	1·30	1·32	1·33	1·34	1·35	1·36	1·37
12	1·38	1·39	1·40	1·42	1·43	1·44	1·45	1·46	1·47	1·48
13	1·49	1·50	1·52	1·53	1·54	1·55	1·56	1·57	1·58	1·59
14	1·60	1·61	1·63	1·64	1·65	1·66	1·67	1·68	1·69	1·70
15	1·71	1·72	1·74	1·75	1·76	1·77	1·78	1·79	1·80	1.81
16	1·82	1·83	1·85	1·86	1·87	1·88	1·89	1·90	1·91	1·92
17	1·93	1·94	1·95	1·96	1·97	1·98	1·99	2·01	2·02	2·03

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